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ALTERED RESPONSIVENESS OF ADENYLATE CYCLASE TO ADENOSINE AND OTHER AGENTS IN THE MYOCARDIAL SARCOLEMMA AND AORTA OF SPONTANEOUSLY-HYPERTENSIVE RATS*

MADHU B. ANAND-SRIVASTAVA†
Clinical Research Institute of Montreal, Montreal, Quebec, Canada H2W 1R7

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Abstract—Adenylate cyclase activity was studied in the myocardial sarcolemma and aorta of spontaneously-hypertensive rats (SHR) and their respectively Wistar–Kyoto (WKY) controls. Basal enzyme activity was decreased in the SHR as compared to the WKY group. Adenylate cyclase stimulation by *N*-ethylcarboxamide adenosine (NECA) was significantly lower in the myocardial sarcolemma and aorta of SHR, and this decreased responsiveness was associated with a reduction in the V_{max}. Other agonists, such as isoproterenol (ISO), epinephrine, dopamine (DA), and glucagon, also enhanced myocardial adenylate cyclase activity to various degrees in SHR and WKY, but stimulation ($V_{agonists}/V_{basal}$) was always lower in the SHR. NaF and forskolin (FSK), which activate adenylate cyclase via receptor-independent mechanisms, augmented it in the myocardial sarcolemma of SHR to a lesser extent than in WKY. While the guanine nucleotides GTP and GMP-P(NH)P elevated adenylate cyclase in a concentration-dependent manner in both SHR and WKY, the magnitude of stimulation was significantly lower in the former group. Decreased basal adenylate cyclase activity and responsiveness to adenosine, various hormones, NaF and FSK were observed in SHR of all ages, i.e. from 4 to 24 weeks of age. In addition, basal, hormone-, NaF- and FSK-stimulated adenylate cyclase activity was diminished markedly in the aorta of SHR. These results suggest that, in SHR, not only is basal adenylate cyclase activity decreased but the abilities of adenosine, other hormones and agonists, such as NaF and FSK, to stimulate adenylate cyclase, guanine nucleotide regulatory protein and the catalytic subunit of the cyclase system are also impaired in the myocardial sarcolemma and aorta.

The elevation of blood pressure in essential hypertension is due to a general increase in the resistance of peripheral vessels [1, 2]. A part of this heightened peripheral resistance has been attributed to structural changes in the vessels [3], abnormalities in calcium movements [4], and aberrations in cyclic nucleotide metabolism [5]. It has been suggested that the adenylate cyclase/cAMP system is one of the biochemical mechanisms which participates in the regulation of arterial tone and reactivity [6]. Reduced cAMP levels in cardiovascular tissues have been implicated in the pathogenesis of hypertension [5, 7], and various studies have reported increased [6], decreased [8] or unaltered [9] adenylate cyclase activity in spontaneously-hypertensive rats (SHR‡). Diminished adenylate cyclase activity has been recorded in response to various hormones, such as catecholamines, glucagon, secretin, and vasoactive-intestinal peptide, in myocardial membranes and in the aorta of SHR [9–11].

Adenosine, a physiological vasodilator which modulates cardiovascular functions [12, 13], has not received much attention, and the mechanism(s) through which it regulates cardiac performance is not yet clear. Various mechanisms have been reported to participate in the vasorelaxant action of adenosine. The adenylate cyclase/cAMP system is one of the mediators of the vasorelaxant effect of adenosine [14]. Adenosine interacts with adenylate cyclase at two distinct sites, designated as extracellular "R" or "A" sites and intracellular "P" sites. The "R" or "A" sites have been further subcategorized as "R_a" or "R_i" sites [15] or A_2 or A_1 sites [16], denoting stimulatory or inhibitory effects on adenylate cyclase.

Adenosine has been shown to suppress the release of norepinephrine from adrenergic nerves within the vascular wall and relaxes smooth muscles [17]. This presynaptic inhibitory action appears to be mediated through specific presynaptic purinergic receptors, which are more sensitive to adenosine than to ATP [18] and are possibly of the A_2 subtype [19]. It has been reported that, in perfused mesenteric arteries from SHR, the inhibitory effect of adenosine on norepinephrine release from presynaptic nerve endings is decreased [20]. Konishi and Su [21] have shown recently that the vasorelaxant action of adenosine in the aorta and femoral arteries of SHR is significantly lower than in Wistar–Kyoto (WKY) rats. The involvement of the endothelium in the relaxant effect of adenosine has been reported in

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† Canadian Heart Foundation Scholar. Address for correspondence: Dr. M. B. Anand-Srivastava, Clinical Research Institute of Montreal, 110 Pine Avenue West, Montreal, Quebec, Canada H2W 1R7.

‡ Abbreviations: SHR, spontaneously-hypertensive rats; WKY, normotensive Wistar–Kyoto rats; NECA, *N*-ethylcarboxamide adenosine; GMP-P(NH)P, guanyl-5′-yl-(β-γ-imino)diphosphate; FSK, forskolin; ISO, isoproterenol; and DA, dopamine.

some [21, 22] but not all [23–26] vascular smooth muscles, indicating that it may partially contribute to adenosine-induced vasorelaxation. Since the adenylate cyclase/cAMP system has been shown to be one of the mechanisms through which adenosine exerts its vasorelaxant effect [14], it is most likely that the direct interaction of adenosine with its receptors coupled to adenylate cyclase is attenuated in SHR, and this may be one of the contributing factors to the lower vasorelaxation effect and elevated blood pressure.

We have demonstrated recently the presence of A_2-type adenosine receptors in the myocardial sarcolemma [27] and cultured vascular smooth muscle cells of the aorta [28] and mesenteric arteries [29]. The present studies were undertaken to examine whether (1) adenosine-sensitive adenylate cyclase activity is altered in the myocardial sarcolemma of SHR; (2) other hormone-sensitive adenylate cyclase activity is also influenced; (3) the changes are confined to receptor-coupled responses or whether guanine nucleotide-sensitive enzyme activity and catalytic activity are also affected; and (4) adenylate cyclase activity stimulated by adenosine and other agents is also altered in vascular tissues such as the aorta, where adenosine-induced vasorelaxation is reportedly diminished in hypertension [21].

MATERIALS AND METHODS

Materials. Adenosine deaminase (EC 3.5.4.5), GTP, GMP-P(NH)P[guanyl-5′-yl-(β-γ-imino)diphosphate], cAMP, L-epinephrine bitartrate, L-norepinephrine bitartrate, isoproterenol (ISO) and dopamine (DA) were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A.; creatine kinase (EC 2.7.3.2) and myokinase (EC 2.7.4.3) from Boehringer Mannheim, Montreal, Québec, Canada; forskolin (FSK) from Calbiochem-Behring Corp., San Diego, CA, U.S.A.; [α^{32}P]ATP from Amersham, Oakville, Ontario, Canada; and *N*-ethylcarboxamide adenosine (NECA) from Research Biochemicals Inc., Wayland, MA, U.S.A. Cultured aortic cells were provided by Dr. Stephen C. Pang.

Female SHR and normotensive WKY rats of different ages were purchased from Charles River Canada (St-Constant, Québec, Canada). Their blood pressure, measured by the tail cuff method without anesthesia, was 98.5 ± 3.3 and 146.5 ± 5.9 mm Hg (N = 15) for the WKY and SHR groups respectively (at 12 weeks of age). Body weights were 172.5 ± 1.9 and 196.4 ± 1.9 g respectively.

Isolation of heart sarcolemma. After acclimatization for a few days, age-matched control WKY and SHR were decapitated, and their hearts were quickly removed and placed in ice-cold 10 mM Tris–HCl buffer (pH 7.4). The heart sarcolemma was isolated essentially according to the method described elsewhere [27]. The ventricles were washed thoroughly, cut into small pieces, and homogenized in a Virtis blender for 30 sec in 10 vol. of 10 mM Tris–HCl buffer (pH 7.4) containing 1 mM EDTA. The homogenate was filtered through cheesecloth and centrifuged at 1000 *g* for 10 min. The sediment was suspended in 20–25 vol. of 10 mM Tris–HCl buffer (pH 7.4), stirred in a cold room for 30 min, and re-centrifuged at 1000 *g* for 10 min. This process was repeated two more times, first by suspending the sediment in 10 mM Tris–HCl buffer at pH 8.0, and then in the same buffer but at pH 7.4. The sediment was again suspended in 20–25 vol. of 10 mM Tris–HCl (pH 7.4), extracted with 0.4 M LiBr for 45 min, and centrifuged at 1000 *g* for 10 min. It was then suspended in 10 mM Tris–HCl (pH 7.4), stirred for 15–20 min, and centrifuged at 1000 *g* for 10 min. The sarcolemmal fraction thus obtained by suspension in a buffer containing 10 mM Tris–HCl, 1 mM dithiothreitol (DTT) and 1 mM EDTA (pH 7.4) was used for the adenylate cyclase determination.

Preparation of aorta-washed particles. Aorta-washed particles were prepared as described previously [30]. The dissected aortae were quickly frozen in liquid nitrogen and pulverized to a fine powder, using a percussion mortar cooled in liquid nitrogen. They were stored at −70° until assayed. After homogenization in a motor-driven Teflon–glass homogenizer in a buffer containing 10 mM Tris–HCl, 1 mM EDTA and 1 mM DTT (pH 7.5), the homogenate was centrifuged at 16,000 *g* for 10 min. The supernatant fraction was discarded, and the pellet was finally suspended in 10 mM Tris–HCl, 1 mM EDTA and 1 mM DTT (pH 7.5), and used for the determination of adenylate cyclase activity.

Adenylate cyclase activity determination. Adenylate cyclase activity was determined by measuring [^{32}P]cAMP formation from [α^{32}P]ATP, as described previously [26, 28]. The incubation assay medium contained 50 mM glycylglycine (pH 7.5), 0.5 mM MgATP or otherwise as indicated, [α-^{32}P]ATP (1 to 1.5×10^6 cpm), 5 mM $MgCl_2$ or otherwise as indicated, 0.5 mM cAMP, 5 units adenosine deaminase/ml, 1 mM DTT, 10 μM GTP, and an ATP-regenerating system consisting of 2 mM creatine phosphate, 0.1 mg creatine kinase/ml, and 0.1 mg myokinase/ml in a final volume of 200 μl. The incubations were initiated by the addition of the sarcolemmal fraction or aorta-washed particles (50–100 μg) to the reaction mixture which had been equilibrated for 2 min at 37°. The reactions were conducted in triplicate for 10 min at 37° and terminated by the addition of 0.6 ml of 120 mM zinc acetate. The other nucleotides were precipitated by the addition of 0.5 ml of 144 mM Na_2CO_3, and [^{32}P]cAMP in the supernatant fraction was purified by using a double column system as described by Salomon *et al.* [31]. Under the assay conditions used, adenylate cyclase activity was linear with respect to protein concentration and time of incubation. Protein was determined essentially by the method of Lowry *et al.* [32], employing crystalline bovine serum albumin as standard.

RESULTS

Effects of various agonists on adenylate cyclase activity in myocardial sarcolemma. Figure 1 shows that epinephrine, ISO, DA and glucagon all enhanced adenylate cyclase activity to various degrees in the myocardial sarcolemma of both SHR and control WKY rats, but the extent of stimulation

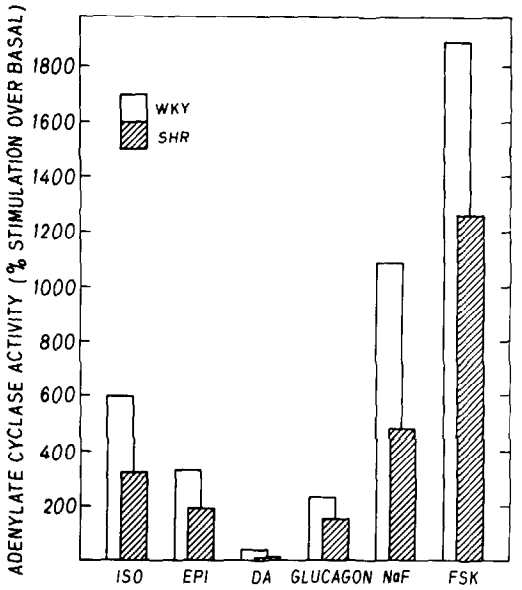

Fig. 1. Effects of various agonists on myocardial adenylate cyclase in 12-week-old WKY (□) and age-matched SHR (▨). The activity of the enzyme was determined in the absence or presence of 50 μM isoproterenol (ISO), 50 μM epinephrine (EPI), 100 μM dopamine (DA), 1 μM glucagon, 10 mM sodium fluoride (NaF) and 50 μM forskolin (FSK), as described in Materials and Methods. The values are the means of triplicate determinations from one of four separate experiments. The SEMs were too small (between 1 and 6 pmol) to be shown in the figure and, therefore, were omitted. Six animals were grouped for each experiment. Basal adenylate cyclase activities in WKY and SHR were 66 ± 6 and 45 ± 3 pmol cAMP (mg protein · 10 min)$^{-1}$ respectively.

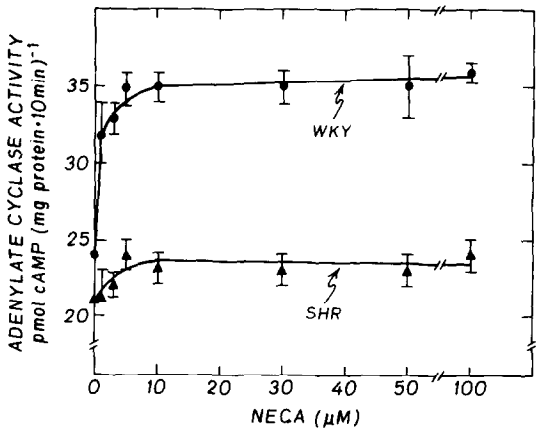

Fig. 2. Effect of various concentrations of NECA on myocardial adenylate cyclase in 12-week-old WKY (●——●) and age-matched SHR (▲——▲). The activity of the enzyme was determined in the presence of 0.2 mM $MgCl_2$ and 1 mM MgATP, as described in Materials and Methods. The values are the means ± SEM of triplicate determinations from one of three separate experiments. Six animals were utilized in each experiment.

was markedly lower in the SHR. ISO and epinephrine were about 45% less effective in SHR than in the WKY group. Similarly, DA stimulated adenylate cyclase activity by about 50% in WKY compared to only 10% in SHR myocardial sarcolemma. NaF and FSK elevated enzyme activity to a lesser degree in SHR (~500% stimulation by NaF in SHR in comparison to 1100% in WKY, and ~1300% stimulation by FSK in SHR relative to ~1900% in WKY). Furthermore, basal adenylate cyclase activity was also lower by about 25–30% in SHR compared to the WKY group. Similar results were obtained with tissues ranging in age from 4, 6, 8, 12, 16, 20 to 24 weeks (data not shown). Decreased basal and hormone-responsive adenylate cyclase activities have also been reported in different models of hypertension by several investigators [8–11, 33].

NECA-sensitive adenylate cyclase in heart sarcolemma from WKY and SHR. As illustrated in Fig. 2, NECA increased adenylate cyclase activity in both SHR and WKY rats in a concentration-dependent manner. However, the degree of stimulation was significantly lower in SHR (~80%) compared to WKY. At 1 μM, NECA enhanced the activity of the enzyme by about 55% in WKY rats, whereas only 10% stimulation was observed in the SHR. The lower effect of NECA in SHR appeared to be associated with a decrease in V_{max} and not with an increase in K_a, suggesting that, in this animal species, the number of adenosine receptors coupled to adenylate cyclase may be reduced.

Effects of guanine nucleotides. Guanine nucleotides are known to regulate adenylate cyclase through their interaction with guanine nucleotide regulatory proteins. To investigate if guanine nucleotide-sensitive adenylate cyclase activity is also impaired in SHR, the effects of GTP and its stable analog GMP-P(NH)P were studied in the myocardial sarcolemma. These results are depicted in Fig. 3. GTP and GMP-P(NH)P stimulated adenylate cyclase in a concentration-dependent manner in both WKY and SHR but the extent of stimulation was markedly lower in SHR. At 1 μM, GTP augmented enzyme activity by about 90% in WKY, whereas only about 65% stimulation was noted in SHR. Similarly, GMP-P(NH)P increased adenylate cyclase in SHR to a lesser degree than in WKY rats. These data indicate that guanine nucleotide regulatory protein, a site for GTP or GMP-P(NH)P action, is also affected in SHR.

Effect of FSK. FSK has been shown to enhance adenylate cyclase activity via a receptor-independent mechanism [34]. Stimulation appears to be mediated by its direct interaction with the catalytic subunit or a component closely associated with it [34, 35]. To ascertain if the catalytic subunit of the enzyme is also impaired in spontaneous hypertension, the effect of FSK on adenylate cyclase was studied in SHR and WKY rats. The results are shown in Fig. 4. FSK elevated adenylate cyclase activity in a dose-dependent manner in both WKY and SHR sarcolemma. However, the extent of stimulation was about 25% lower in the latter at all FSK concentrations used.

NECA-sensitive adenylate cyclase activity in aorta of WKY and SHR. Since the aorta and femoral arteries of SHR present a lesser relaxant response to adenosine than those of WKY rats [21], it seemed interesting to examine if this lower vasorelaxation is

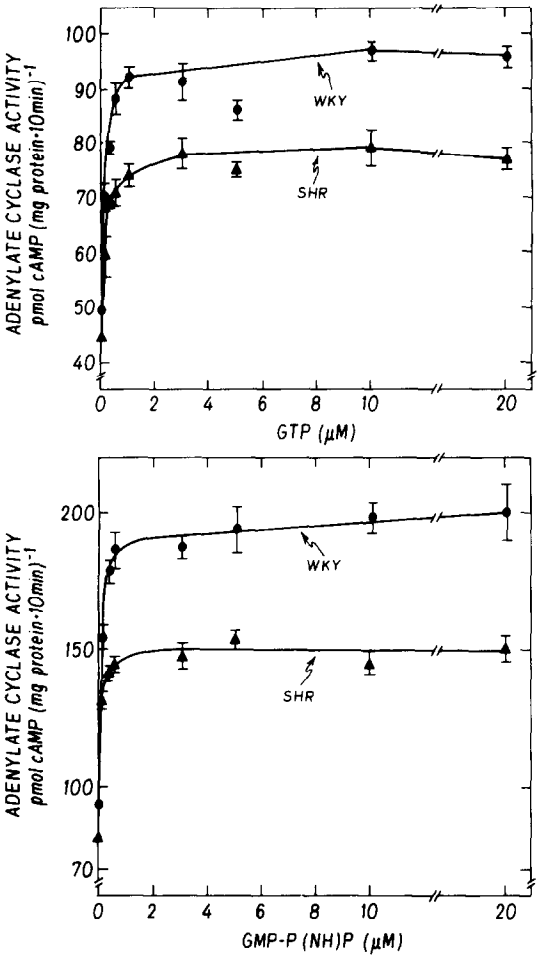

Fig. 3. Effects of guanine nucleotides on myocardial adenylate cyclase in 12-week-old WKY (●——●) and age-matched SHR (▲——▲). Adenylate cyclase activity was determined in the absence or presence of various concentrations of GTP (upper panel) or GMP-P(NH)P (lower panel), as described in Materials and Methods. The values are the means ± SEM of triplicate determinations from one of three separate experiments. Eight animals were used in each experiment.

the consequence of diminished adenylate cyclase stimulation by adenosine. Figure 5 reveals that NECA enhanced adenylate cyclase activity in a concentration-dependent manner with an apparent K_a between 0.5 and 1 μM in both WKY and SHR. However, the extent of stimulation was significantly lower in SHR. NECA, at 1 μM, raised the activity of the enzyme by about 7-fold in WKY compared to the 3.5-fold stimulation seen in SHR. The lower enzyme values in SHR appeared to be associated with a decrease in V_{max} and not with an increase in K_a. These data, which are similar to those on heart sarcolemma, indicate that adenosine receptors may also be reduced in the aorta of SHR.

To establish if the abilities of other agents to activate adenylate cyclase through receptor-dependent and -independent mechanisms are also decreased in the aorta of SHR, the effects of ISO, epinephrine, NaF and FSK were studied in these

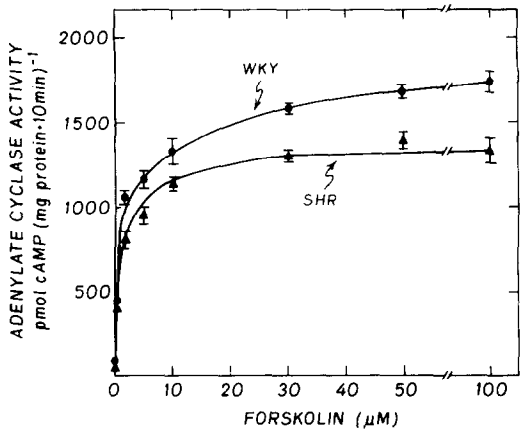

Fig. 4. Effect of various concentrations of forskolin on myocardial adenylate cyclase in 12-week-old WKY (●——●) and age-matched SHR (▲——▲). Adenylate cyclase activity was determined in the absence of GTP as described in Materials and Methods. The values are the means ± SEM of triplicate determinations from one of three separate experiments. Six animals were grouped in each experiment.

animals and their control counterparts, Figure 6 demonstrates that ISO and epinephrine stimulated adenylate cyclase activity by about 75 and 40%, respectively, in the WKY aorta, and by about only 40 and 10% in SHR aorta. In addition, NaF- and FSK-sensitive enzyme activity was about 50% lower in SHR. These findings suggest that adenylate cyclase in the SHR aorta, as in the heart sarcolemma, is less responsive to various hormones and agents that activate adenylate cyclase via receptor-independent mechanisms.

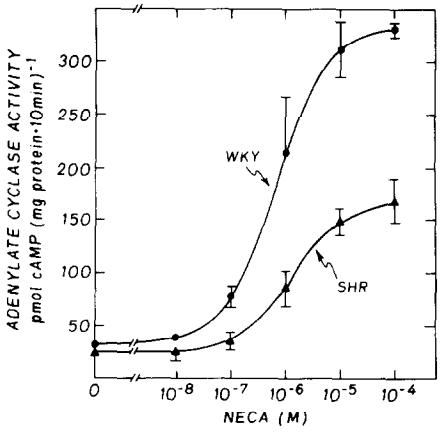

Fig. 5. Effect of various concentrations of NECA on adenylate cyclase activity in cultured vascular smooth muscle cells from the aorta of 12-week-old WKY (●——●) and age-matched SHR (▲——▲). Cells were cultured essentially by the method described previously [28]. Confluent cells were washed, scraped from the petri dishes, and homogenized in a Dounce homogenizer in a buffer containing 10 mM Tris, 1 mM EDTA and 1 mM DTT (pH 7.5). The homogenate was used to determine adenylate cyclase activity, as described in Materials and Methods. The values are the means ± SEM of triplicate determinations from one of three separate experiments. Six animals were utilized in each experiment.

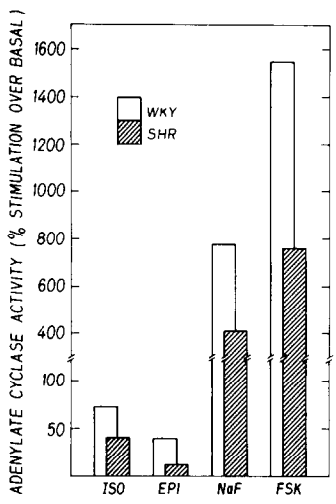

Fig. 6. Effects of various agonists on adenylate cyclase in aorta-washed particles from 12-week-old WKY (□) and age-matched SHR (▨). The activity of the enzyme was determined in the absence or presence of 50 μM isoproterenol (ISO), 50 μM epinephrine (EPI), 10 mM sodium fluoride (NaF) and 50 μM forskolin (FSK), as described in Materials and Methods. The values are the means of triplicate determinations from one of three separate experiments. The SEMs were too small to be shown in the figure and, therefore, were omitted. Eight animals were grouped in each experiment. Basal adenylate cyclase activities in the WKY and SHR were 41 ± 1 and 27 ± 1 pmol cAMP (mg protein · 10 min)$^{-1}$ respectively.

DISCUSSION

The data presented in this paper demonstrate that basal as well as agonist-stimulated adenylate cyclase activity was lower in the myocardial sarcolemma and aorta of SHR in comparison to normotensive control rats. Decreased basal adenylate cyclase activity was observed in all stages of hypertension (4–24 weeks of age); in older animals, it may be partly due to increased ventricular weight with advancing age when hypertrophy of the heart is reported to occur [36]. In the early stages of hypertension, however, the reduction of basal and hormone-sensitive adenylate cyclase activity may be ascribed to an impaired adenylate cyclase system *per se* and not to hypertrophy, because no significant cardiac enlargement is evident [35]. The other possible cause of the observed decrease in basal enzyme activity in SHR may be the increased levels of circulating atrial natriuretic factor [37], which inhibits adenylate cyclase in various target tissues [38–41].

The lower stimulation of the enzyme by β-adrenergic agents in the SHR aorta and by β-adrenergic agents, DA, and glucagon in the SHR sarcolemma (as compared to control WKY rats) may be due to the possible reduction in the number of hormone receptors coupled to adenylate cyclase; and/or guanine nucleotide regulatory protein, which couples the receptors to the catalytic subunit of adenylate cyclase, may be defective; or the hormone receptors may be desensitized because of the elevated levels of circulating hormones [5] in SHR. A diminution of catecholamine-sensitive adenylate cyclase activity associated with a decrease in the number of β-adrenergic receptors has been demonstrated in renal and deoxycorticosterone acetate-salt hypertensive rats [42] as well as in SHR [36]. In our present studies, we have shown that the abilities of guanine nucleotides, such as GTP and GMP-P(NH)P, to stimulate adenylate cyclase were decreased in the SHR sarcolemma, indicating that guanine nucleotide regulatory protein, a site of GTP action, is impaired in SHR. It may be one of the factors contributing to the observed decrease in hormone-sensitive adenylate cyclase activity.

We have reported previously the presence of adenosine-stimulatory receptors coupled to adenylate cyclase in the heart sarcolemma and cultured vascular smooth muscle cells of the aorta and mesenteric arteries [27–29]. In the current studies, it was demonstrated that adenosine-sensitive adenylate cyclase activity was decreased in the SHR sarcolemma and aorta. Since the diminished stimulation of adenylate cyclase by NECA appeared to be associated with a fall in V_{max} and not with an increase in K_a in both the sarcolemma and aorta, it is possible that the adenosine receptors coupled to adenylate cyclase may also be reduced in SHR. A similar decrease has been reported in the responsiveness of adenylate cyclase to adenosine in the myocardial sarcolemma of renal hypertensive rats [33].

A lower vasorelaxant effect of adenosine on vascular smooth muscles has been demonstrated in SHR versus WKY rats [21]. In addition, the sensitivity of perfused mesenteric blood vessels to adenosine has been noted to be six times lower in SHR than in WKY [21]. Taken together, it may be suggested that the observed decrease in adenosine-induced vasorelaxation in SHR may be due to the reduced formation of cAMP by adenosine in SHR. However, the lower inhibition of catecholamine release by adenosine from presynaptic nerve endings in SHR [20] cannot be ignored and may be another possible mechanism responsible for the lesser vasorelaxant effect of adenosine in SHR.

FSK, a positive inotropic and antihypertensive agent [43], has been shown to activate adenylate cyclase in several tissues [34]. In the present studies, the stimulatory effects of NaF and FSK on the enzyme were inhibited in SHR, indicating that the catalytic subunit may also be impaired in SHR. Decreased adenylate cyclase sensitivity in SHR aorta and the total lack of its response to NaF in the aorta of stress-induced hypertensive rats have been reported previously [9]. The diminished stimulation of adenylate cyclase by NaF and FSK in SHR may be due to the impairment of guanine nucleotide regulatory protein which has been shown to be involved in the adenylate cyclase activation by NaF and possibly by FSK. In addition, phospholipids have also been found to be required for the expression of hormone-, NaF- and FSK-stimulated adenylate cyclase activity [44, 45]. Any alteration in the phospholipid composition of membranes may result in a loss or diminished responsiveness of the enzyme to these agents, and such changes in membrane phospholipid composition may occur in hypertension.

In conclusion, adenylate cyclase responsiveness to adenosine and other hormones was decreased in the aorta and heart sarcolemma of SHR. The reduced formation of cAMP by adenosine in the SHR aorta and heart sarcolemma may be one of the mechanisms responsible for the respective diminution of vaso-relaxation and impaired myocardial function in hypertension.

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REFERENCES

1. C. M. Ferrario and I. H. Page, *Circulation Res.* **43**, 821 (1978).
2. A. C. Guyton, T. G. Coleman and H. J. Granger, *Physiol. Rev.* **52**, 13 (1972).
3. B. Folkow, M. Hallback, V. Lundgren, R. Sivertsson and L. Weiss, *Circulation Res.* **32**(Suppl. I), 1 (1973).
4. E. E. Daniel, in *Vasolidators* (Eds. P. M. Vanhoutte and I. Leusen), p. 381. Raven Press, New York (1981).
5. M. S. Amer, *Life Sci.* **17**, 1021 (1975).
6. L. Triner, Y. Vulliemoz, M. Verosky, D. V. Habif and V. V. Nahas, *Life Sci.* **11**, 817 (1972).
7. S. Ramanathan, S. Shibata, T. K. Tashaki and R. N. Ichord, *Biochem. Pharmac.* **25**, 233 (1976).
8. S. Ramanathan and S. Shibata, *Blood Vessels* **11**, 312 (1974).
9. M. S. Amer, *Science* **179**, 807 (1973).
10. P. Chetalain, P. Robberecht, P. De Neef, J. C. Camus, D. Heuse and J. Christopher, *Pflügers Archs* **389**, 29 (1980).
11. G. C. Palmer and S. Greenberg, *Pharmacology* **19**, 156 (1979).
12. A. N. Drury and A. Szent-Gyorgyi, *J. Physiol. Lond.* **68**, 213 (1929).
13. R. M. Berne, *Am. J. Physiol.* **204**, 317 (1963).
14. W. P. Kukovetz, G. Pöch, S. Holtzmann, A. Wurm and I. Rinner, *Adv. Cyclic Nucleotide Res.* **9**, 397 (1978).
15. C. Londos, D. M. F. Cooper and J. Wolf, *Proc. natn. Acad. Sci. U.S.A.* **77**, 2551 (1980).
16. D. Van Calker, M. Muller and B. Hamprecht, *J. Neurochem.* **33**, 999 (1979).
17. C. Su, *A. Rev. Pharmac.* **23**, 397 (1983).
18. G. Burnstock, in *Purinergic Receptors* (Ed. G. Burnstock), p. 1. Chapman & Hill, London (1981).
19. C. Kennedy and G. Burnstock, *Eur. J. Pharmac.* **100**, 363 (1984).
20. Y. Kamikawa, W. H. Cline and C. Su, *Eur. J. Pharmac.* **66**, 347 (1980).
21. M. Konishi and C. Su, *Hypertension* **5**, 881 (1983).
22. G. Rubanyi and P. M. Vanhoutte, *J. cardiovasc. Pharmac.* **7**, 139 (1985).
23. J. G. De Mey and P. M. Vanhoutte, *J. Physiol., Lond.* **316**, 347 (1981).
24. T. D. White, A. Chandhry, M. M. Vohra, D. Webb and R. Leslie, *Eur. J. Pharmac.* **118**, 37 (1985).
25. J. G. De Mey and P. M. Vanhoutte, *Circulation Res.* **51**, 439 (1982).
26. C. Kennedy, D. Delbro and G. Burnstock, *Eur. J. Pharmac.* **107**, 101 (1985).
27. M. B. Anand-Srivastava, *Archs Biochem. Biophys.* **243**, 439 (1985).
28. M. B. Anand-Srivastava, D. J. Franks, M. Cantin and J. Genest, *Biochem. biophys. Res. Commun.* **108**, 213 (1982).
29. M. B. Anand-Srivastava and D. J. Franks, *Life Sci.* **37**, 857 (1985).
30. M. B. Anand-Srivastava, *Biochem. biophys. Res. Commun.* **117**, 420 (1983).
31. Y. Salomon, C. Londos and M. Rodbell, *Analyt. Biochem.* **58**, 541 (1974).
32. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
33. M. B. Anand-Srivastava, M. Cantin and J. Genest, *Biochem. Pharmac.* **32**, 2857 (1983).
34. K. B. Seamon and J. W. Daly, *J. Cyclic Nucleotide Res.* **7**, 201 (1981).
35. P. A. Insel, D. Stengel, N. Ferry and J. Hanoune, *J. biol. Chem.* **257**, 7485 (1982).
36. R. C. Bhalla, R. V. Sharma and S. Ramanathan, *Biochim. biophys. Acta* **632**, 497 (1980).
37. J. Gutkowska, K. Horky, C. Lachance, K. Racz, R. Garcia, G. Thibault, O. Kuchel, J. Genest and M. Cantin, *Hypertension* **8**(Suppl. I), I–137 (1986).
38. M. B. Anand-Srivastava, D. J. Franks, M. Cantin and J. Genest, *Biochem. biophys. Res. Commun.* **121**, 855 (1984).
39. M. B. Anand-Srivastava, M. Cantin and J. Genest, *Fedn Eur. Biochem. Soc. Lett.* **181**, 199 (1985).
40. M. B. Anand-Srivastava, M. Cantin and J. Genest, *Life Sci.* **36**, 1873 (1985).
41. M. B. Anand-Srivastava, P. Vinay, J. Genest and M. Cantin, *Am. J. Physiol.* **251**, F417 (1986).
42. E. A. Woodcock, J. W. Funder and C. I. Johnston, *Circulation Res.* **45**, 560 (1979).
43. E. Lindner, A. N. Dohadwalla and B. K. Bhattacharya, *Arzneimittel-Forsch.* **28**, 284 (1978).
44. M. B. Anand-Srivastava and M. Cantin, *Pharmacologist* **25**, 141 (1983).
45. M. B. Anand-Srivastava and R. A. Johnson, *J. Neurochem.* **36**, 1819 (1981).